Bull. Environ. Contam. Toxicol. (1986) 37:281–288

Bulletin of Environmental Contamination and Toxicology

Acute Toxicity of Methyl Mercury to the Larval Lamprey, *Petromyzon marinus*

J. Mallatt,[1] M. G. Barron,[2] and C. McDonough[1]

[1]Department of Zoology and [2]Program in Pharmacology/Toxicology, Washington State University, Pullman, WA 99164-4220

Mercury compounds pollute many aquatic habitats and are extremely toxic to aquatic organisms. In fish, mercury accumulates as methyl mercury, which is taken up from food, and especially from the water (Jernelov and Lann 1971). Acute toxicity of waterborne methyl mercury has been studied in several teleost species (Matida et al. 1971; Roales and Perlmutter 1974; Wobeser 1975; McKim et al. 1976; Lock et al. 1981; Sharp and Neff 1982). Lampreys are taxonomically distant from teleosts and we utilize them for comparative toxicological purposes. Landlocked sea lampreys, Petromyzon marinus, inhabit the Great Lakes region, and their larvae (ammocoetes) burrow in stream sediments. In this study, we report toxicity curves for ammocoetes exposed acutely to methyl mercuric chloride solutions. Susceptibility was related to temperature and animal size.

MATERIALS AND METHODS

About two hundred larvae, 1-3 years old, were collected from streams in Northern Michigan by crews of the Marquette Biological Station of the U.S. Fish and Wildlife Service. The animals were shipped directly to our laboratory and held at 12° for 1-6 months before use in toxicity tests. They were fed yeast suspensions.

Test animals ranged from 5-12.5 cm in length and from 0.3-3.0 g in weight, with a weight-length relation of $W = 2.7 \times 10^{-3} L^{-2.7}$ ($r = .98$). A condition factor (CF) measured thinness: $W \times L^{-2.54} \times 10^{5}$. Only non-emaciated larvae with CF>330 were used in this study, and 80% of the larvae had CF>350 . This matched the condition of the animals as received from the wild.

Dechlorinated tap water was used for holding animals and for toxicant exposure. Water chemistry parameters were: pH 8.0-8.5; total hardness 145 mg $CaCO_3$/L; alkalinity 150 mg CaCO/L; sodium 23 mg/L;

copper <1 µg/L; ammonia 0.01 mg N/L; nitrates 0.03 mg N\L; nitrites 0.01 mg N/L; total phosphorus 0.035 mg/L; specific conductance 320 µM/cm. The water was dechlorinated by vigorous aeration for 50 to 72 hours.

Toxicant was administered with an Ace Flow-Thru Bioassay Diluter System^{R} (Ace Glass, Vineland, New Jersey). The system was set up to deliver six toxicant concentrations, with each concentration about 60% as great as the previous, to a series of seven glass 3.5 L exposure vessels (no replicates). The seventh (control) vessel received pure water. Solution in each vessel was renewed at a rate of 33-43% per hour, so 90% replacement occurred every six hours or less.

Methyl mercury chloride was added to the diluter from an aqueous stock solution (3-4 g Hg/L). Methyl mercury concentration in test water was measured as µg Hg/L by the cold vapor atomic absorption method (APHA 1975: Perkin-Elmer Atomic Absorption spectrophotometer) after the water samples had been acidified <pH 2 with nitric acid. $HgCl_2$ solutions were used as standards.

Four toxicity tests were performed, sequentially not simultaneously, yielding toxicant concentrations listed in Table 1. The 12° test was replicated, but the 4° and 20° tests were not, due to a limited supply of animals.

Table 1. Exposure conditions

Temp.	Toxicant Conc. (µg Hg/L)	S.D.[b]	Exposure
4° C.	214,113,82,62,30,0.3[a]	6%	9 days
12° C (first)	104,48,29,17,9,2.5,0.2[a]	20%	14 days
12° C (second)	166,88,52,32,18,3.2,0.2[a]	6%	12 days
20°	118,90,56,34,18,0.3[a]	8%	10 days

[a] lowest concentration represents control vessels.
[b] mean standard deviation of Hg conc. in the vessels

Larvae to be used in 4° and 20° tests were acclimated gradually to these temperatures (1° per day) and then held twenty more days before being exposed to toxicant. Temperature never varied >0.5° from the desired.

Eighty hours before each test, larvae were moved to the room containing the exposure apparatus and placed in acclimation tanks, with sand substrate. Feeding was halted 24 hours before toxicant exposure, sufficient time for the gut to empty (Mallatt, unpublished).

The tests began as animals were thrust into toxicant solutions, concentrations of which had been allowed to stabilize. Animals were assigned randomly to test vessels. For each 12^{o} test, there were ten larvae per vessel. At 4^{o} and 20^{o}, seven larvae occupied each vessel. Total fish biomass was below 0.3 g per liter of solution passing through the vessels per day. Artificial substrate for burrowing (EPA 1975) was a layer of small pieces of Tygon^{R} tubing (200 ml), overlain by a 0.1 m^2 piece of fiber glass screen. Except for brief periods of checking for mortality, lights remained off during exposures, as ammocoetes are calmed by darkness. Mortality (no response to gentle prodding) was assessed thrice daily, or more often as needed. Dead animals were removed and measured.

Water quality was monitored in the test vessels at 2-3 day intervals throughout the exposure period: Oxygen was always >70% saturated; pH stayed at 8.3-8.5; ammonia and nitrite remained undetectable, i.e., below 1 and 0.1 mg N/L, respectively. Mercury levels were measured in all vessels on the first and second day of exposure, then every two to three days thereafter.

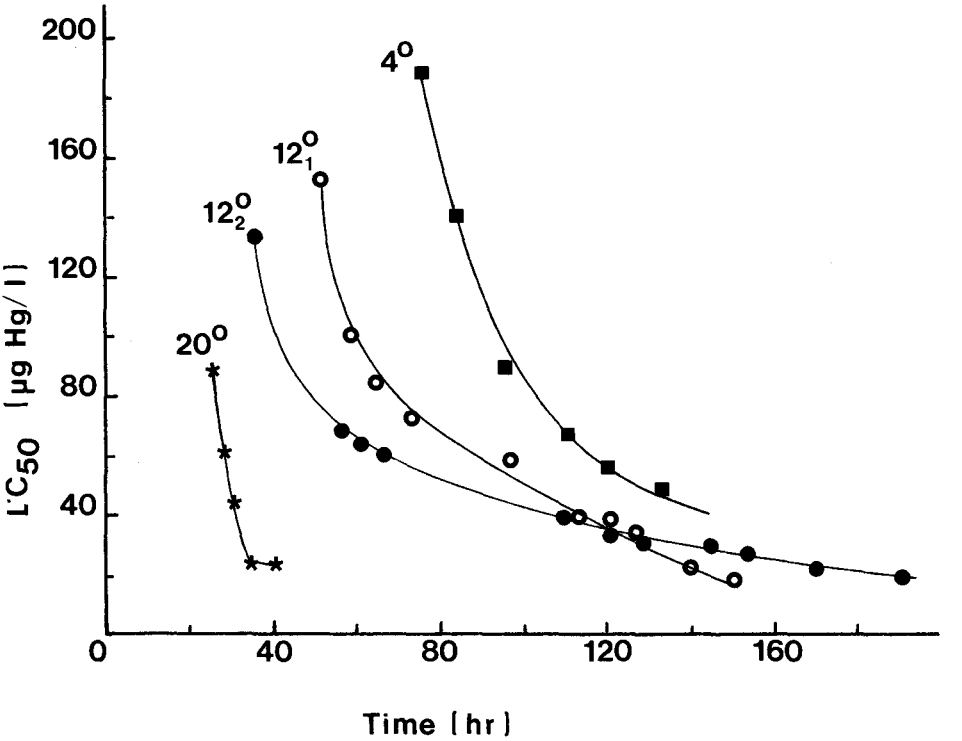

Figure 1. Toxicity curves for lamprey larvae exposed to methyl mercury at three different temperatures (4,12,20^{o} C), with test replicated at 12^{o}. Curves were fit to points by eye.

Table 2. LC50 values (μg Hg/L) for larval Petromyzon marinus exposed to methyl mercury solutions at various temperatures (95% confidence intervals given in parentheses).

	24 h	36 h	48 h	72 h	96 h	120 h	144 h	168 h
4°				191 (150-301)	88 (75-109)	57 (37-77)		
12° (#1)			151 (100-174)	76 (63-96)	62 (40-155)	40 (21-140)	20 (16-25)	
(#2)	>166	126[a] (100-150)	88[a] (68-104)	58[a] (48-74)	48[a] (38-57)	33 (26-42)	29 (21-37)	26 (20-32)
20°	90 (73-113)	34[b]						

[a] Fewer than two partial kills had occurred at precisely 36, 48, 72, and 96 h in this test, which precluded direct calculation of these LC50 values via probit analysis. The values listed here are interpolations based on the following results: 34-h LC50 = 134 (CI: 110-163); 57-h LC50 = 71 (CI: 59-87); 64-h LC50 = 61 (CI: 51-75); 109-h LC50 = 41 (CI: 34-51).

[b] This 36-h LC50 value at 20° is an estimate based on the binomial method. Probit analysis puts a 29-h LC50 at 44 μg Hg/L, with a confidence interval of 32-55.

LC50 values were calculated by probit analysis. Also explored was the relationship between order of death and animal length; here, mortality data for all test vessels were pooled for each temperature and evaluated with a Spearman rank correlation test. Calculations used the SAS computer program (SAS Institute 1982).

RESULTS AND DISCUSSION

Ammocoetes exposed to methyl mercury above 10 µg/L seemed slightly narcotized. Some exhibited spontaneous bouts of spastic swimming when near death. No control animal died in any of the four toxicity tests. Figure 1 shows the toxicity curves for methyl mercury exposure at 4, 12, and 20°. Table 2 summarizes LC50 values obtained in the tests.

Table 3 shows results of the rank correlation test. In three of the four toxicity tests, there was no relation between animal size and order of death; a relation was suggested in one test, however (first 12° test). Noting the exception, we interpret the data as demonstrating no clear association between animal size and susceptibility to methyl mercury.

Table 3. Spearman test for correlation (r_s) between animal size rank and order of death. A P-value of >0.05 implies no relation.

Temperature	r_s	P	average weight,g.	#dead
4°	0.15	0.38	0.75±0.4 SD	34
12° (#1)	-0.50	0.01	0.73±0.4 SD	29
12° (#2)	0.07	0.65	0.70±0.3 SD	40
20°	0.10	0.55	0.75±0.4 SD	33

Due to a seasonal unavailability of lampreys, we were limited to only 7-10 animals per exposure vessel in this study. Despite this suboptimal sample size, the variability of our results was not excessive, except in the first 12° exposure. There, some LC50 values had large confidence intervals (Table 2); we suspect, however, that this uncertainty resulted less from low animal number than from the considerable fluctuation of mercury level during this test (average standard deviation of 20%: Table 1). Despite any oddities in the first 12° test, the toxicity curves for the two 12°

tests do not differ much: their 95% confidence intervals overlap for all LC50 values (Table 2).

Our results indicate the susceptibility of ammocoetes to methyl mercury increases with temperature (Figure 1). Rehwoldt et al. (1972) noted the same in teleosts exposed to $HgCl_2$, as did Heit and Fingerman (1977) in crayfish. The reason for the temperature dependency of mercury toxicity is not known, although Reinert et al. (1974) found methyl mercury uptake rate from water to increase modestly with temperature in Salmo gairdneri.

While temperature influences methyl mercury toxicity to lampreys at high toxicant concentrations, the incipient lethal concentration (ILC: maximum concentration producing no acute mortality) may be a constant, unaffected by temperature. At the right side of Figure 1, all the toxicity curves seem to converge asymptotically toward a single mercury concentration. This ILC cannot be estimated precisely from the graph, but we have additional evidence placing it close to 10 µ g Hg/L: In a separate test at 12^{o}, we exposed eleven ammocoetes to methyl mercury at 10 µg Hg/L for 21 days, and obtained just one death in that period. Likewise, at 20^{o}, we have evidence that the toxicity curve levels off around 10 µg Hg/L: Three larvae were held in a methyl mercury solution of 8 µg Hg/L for eight days, without mortality. Sprague (1985) concluded temperature tends not to affect incipient lethal concentrations of most toxicants to fish. This is consistent with our findings.

Our study revealed no relationship between ammocoete size and susceptibility to methyl mercury (Table 3). Perhaps such a relationship would emerge if a wider range of sizes were used, including ammocoetes younger than one year. Studies suggesting that methyl mercury toxicity to aquatic animals varies with animal size (or age) include: Wobeser (1975), McKim et al.(1976), and Heit and Fingerman (1977).

How do ammocoetes compare to teleosts in susceptibility to methyl mercury? Past studies used many different temperatures and water conditions, so results are difficult to compare. Even so, data of Table 4 suggest different fish are quite uniform in susceptibility to methyl mercury--and lampreys are not atypical in their susceptibility. The methyl mercury LC50 for 24 hours is higher in lampreys than in any other fish for which this value is known. Perhaps this relates to the exceptionally low metabolic rates of ammocoetes (Lewis, 1980), which might slow the rate at which uptaken mercury can disrupt physiological processes.

Table 4. Summary of LC50 values (µg Hg/L) for various fish exposed to waterborne methyl mercury.

Study	24 h	48 h	96 h
Present study			
Petromyzon marinus	>166	88	48
12 °C, 0.3-3 g fish			
hardness: 146 mg $CaCo_3$/L			
Matida et al. (1971)			
Salmo gairdneri	52	37	31
17 °C, 5 g fish			
Wobeser (1975)			
S. gairdneri fry	84	45	24
S. gairdneri fingerlings	125	66	42
10 °C, up to 3 g fish			
hardness: 100 mg $CaCo_3$/L			
Lock et al. (1981)			
S. gairdneri	-	-	24
12 °C, 18-23g fish			
McKim et al. (1976)			
Salvelinus fontinalis			
12 °C, 120 g fish	-	-	65
12 °C, 12 g fish	-	-	84
hardness: 45 mg $CaCo_3$/L			
Roales and Perlmutter (1974)			
Trichogaster trichopterus	123[a]	94[a]	90[a]
27 °C, 2 g fish			
Sharp and Neff (1982)			
Fundulus heteroclitus	-	-	50-100+
25 °C, embryos; NOTE: Brackish water			

[a]unclear if concentrations were presented as Hg or as methyl-Hg.

Acknowledgments. This work was funded by a grant from the National Institutes of Health (1 ROl ESO3240-01). Thanks are extended to Harry Purvis, and Richard Torblaa for supplying animals, and to Ervin Hindin, and Charles Paulsen for help with water analysis and mercury measurements.

REFERENCES

American Public Health Association (1975) Standard methods for the examination of water and waste-water, 14th ed., pp 156-159

Environmental Protection Agency (1975) Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. EPA-660/3-75-009. 61 pp

Heit M, Fingerman M (1977) The influences of size, sex and temperature on toxicity of mercury to two species of crayfishes. Bull Environ Contam Toxicol 18:572-580

Jernelov A, Lann H (1971) Mercury accumulation in food chains. Oikos 22:403-406

Lewis SV (1980) Respiration of lampreys. Can J Fish Aquat Sci 37:1711-1722

Lock R, Cruijsen P, van Overbeeke A (1981) Effects of mercuric chloride on the osmoregulatory function of the gills in rainbow trout, Salmo gairdneri Richardson. Comp Biochem Physiol 68C:151-159

Matida Y, Kumada H, Kimura S, Saiga Y, Nose T, Yokote M, Kawatsu H (1971) Toxicity of mercury compounds to aquatic organisms and accumulation of the compounds by the organisms. Bull Freshwater Fish Res Lab (Japan) 21:197-227

McKim JM, Olson GF, Holcombe GW, Hunt EP (1976) Long-term effects of methylmercuric chloride on three generations of brook trout (Salvelinus fontinalis): toxicity, accumulation, distribution, and elimination. J Fish Res Bd Can 33:2726-2739

Rehwoldt R, Menapace LW, Nerrie B, Alessandrello D (1972) The effect of increased temperature upon the acute toxicity of some heavy metal ions. Bull Environ Contam Toxicol 8:91-96

Reinert RE, Stone LJ, Willford WA (1974) Effect of temperature on accumulation of methylmercuric chloride and p,p´DDT by rainbow trout (Salmo gairdneri). J Fish Res Bd Can 31:1649-1652

Roales RR, Perlmutter A (1974) Toxicity of methylmercury and copper, applied singly and jointly, to the blue gourami, Trichogaster trichopterus. Bull Environ Contam Toxicol 12:633-639

SAS Institute (1982) SAS user´s guide: statistics. SAS Institute, Inc. Cary, North Carolina. 584 pp

Sharp JR, Neff JM (1982) The toxicity of mercuric chloride and methylmercuric chloride to Fundulus heteroclitus embryos in relation to exposure conditions. Env Biol Fish 7:277-284

Sprague JB (1985) Factors that modify toxicity. In: Rand GM and Petrocelli SR (eds) Fundamentals of aquatic toxicology. Hemisphere, New York, pp 124-163.

Wobeser G (1975) Acute toxicity of methyl mercury chloride and mercuric chloride for rainbow trout fry and fingerlings. J Fish Res Bd Can 32:2005-2013

Received July 19, 1985; accepted October 26, 1985